# *Bio-efficacy of hydroxy-selenomethionine as a selenium supplement in pregnant dairy heifers and on the selenium status of their calves*

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# Bioefficacy of hydroxy-selenomethionine as a selenium supplement in pregnant dairy heifers and on the selenium status of their calves

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## ABSTRACT

This study aimed to determine the effects of supplementing pregnant heifers with the organic selenium (Se) source 2-hydroxy-4-methylselenobutanoic acid (HMSeBA) during the last 8 wk of pregnancy on dam and calf Se status. A total of 42 in-calf heifers were recruited to the study and randomly allocated to 1 of 3 treatments; a negative control (Con), sodium selenite (NaSe), or HMSeBA. Animals were blocked by body weight, body condition score, and expected calving date before treatment allocation. Following enrollment, all animals underwent a 7-wk wash-out period, after which they received their respective supplements, top-dressed daily onto a basal diet for the last 8 wk of pregnancy. Heifer blood samples were taken at weekly intervals from enrollment until 2 wk before expected calving date and as soon as possible after calving for determination of whole-blood glutathione peroxidase activity (GSH-Px) and plasma Se and malondialdehyde (MDA) concentrations. Selenized AA were determined in plasma samples taken at 3 wk precalving. A colostrum sample was taken as close to parturition as possible for determination of colostrum total Se, selenized AA, and IgG concentration. Calves were blood sampled as close to birth as possible for determination of whole-blood GSH-Px activity and plasma Se and MDA concentrations. Differences in whole-blood GSH-Px activity did not become apparent until calving; GSH-Px activity was lowest in Con heifers but similar between NaSe and HMSeBA heifers. Plasma Se was lowest in unsupplemented heifers and greatest in those supplemented with HMSeBA; this was attributable to greater selenomethionine concentrations in the plasma of HMSeBA heifers. Colostrum Se was lowest in Con heifers and greatest in HMSeBA heifers. The greater Se concentration of HMSeBA heifers was attributable to a greater proportion of total Se comprising selenocysteine; the reason for this is not known. There was no effect of supplementation on colostrum IgG concentration. Plasma Se was lowest in calves born to Con heifers and greatest in those born to HMSeBA heifers. There were no effects of treatment on calf whole-blood GSH-Px activity or plasma MDA concentration. The enhanced Se status associated with HMSeBA supplementation is likely a consequence of selenomethionine supply and may confer benefits to both the dam and her calf postpartum.
**Key words:** selenium, selenomethionine, heifer, calf

## INTRODUCTION

Selenium (Se) is recognized as an essential nutritional trace element, and since the beginning of this century a considerable body of research has contributed significantly to our understanding of its biological importance, functionality, and uptake and assimilation. The biological actions of Se are undertaken by selenoproteins, which are characterized by the presence of selenocysteine (**SeCys**) residues within their primary structure (Labunskyy et al., 2014; Burk and Hill, 2015). However, selenoproteins should not be confused with Se-containing proteins in which selenomethionine (**SeMet**) has been nonspecifically incorporated in place of methionine within the primary structure of the protein. Once ingested, SeMet is transported across the brush border of the gastrointestinal tract by $Na^+$-dependent methionine transporters (Wolffram et al., 1989) and enters the methionine pool of the body, where it can be later utilized as an endogenous Se supply for synthesis of selenoproteins (Burk and Hill, 2015).

To meet an animal's Se requirements, feeds are usually supplemented with either mineral forms of Se (sodium selenate or selenite) or organic forms, which comprise predominantly SeMet. In monogastric species, the greater bioavailability and efficacy of organic forms compared with mineral forms has been extensively reviewed (Surai and Fisinin, 2016; Surai et al., 2018). Similarly, results obtained from ruminant species have also demonstrated the greater bioefficacy of organic forms compared with mineral forms (Juniper et al., 2006; Phipps et al., 2008; Gong et al., 2014). This

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improved bioefficacy of organic forms is directly related to the SeMet concentration of the Se supplement rather than its total Se concentration.

The Se compound 2-hydroxy-4-methylselenobutanoic acid (**HMSeBA**), or hydroxy-selenomethionine, is an alternative organic Se source, and it too has been shown to be more efficacious than mineral forms when offered to broiler chickens (Briens et al., 2013, 2014), laying hens (Jlali et al., 2013), finishing pigs (Jlali et al., 2014), and dairy cattle (Sun et al., 2017, 2019). Little is known about the actual transport of HMSeBA across the gastrointestinal tract, but studies in poultry have demonstrated that the sulfur homolog of HMSeBA, DL-2-hydroxy-4-(methylthio) butanoic acid, is transported across the apical membrane by $H^+$-dependent carrier-mediated mechanisms related to L-lactate transport (Martín-Venegas et al., 2006). Hydroxy-selenomethionine is a precursor of SeMet and is metabolized in the same way as SeMet (Surai et al., 2018). Briens et al. (2014) reported following speciation analysis of tissues taken from poultry fed diets supplemented with HMSeBA that HMSeBA was undetectable and that the majority of total Se was present as either SeMet or SeCys.

There is a positive correlation between the Se status of the dam at parturition and that of her offspring, and the Se status of calves born to dams supplemented with dietary Se during the latter stages of pregnancy is more effectively maintained in the early postpartum period than that of calves receiving injectable sources of Se (Kincaid and Hodgson, 1989). More recent studies have demonstrated that the supplementation of pregnant cattle with organic forms of Se during the latter stages of pregnancy further improves the Se status of their offspring compared with those that received mineral forms of Se (Pehrson et al., 1999; Gunter et al., 2003; Guyot et al., 2007). The present study aimed to compare the effects of HMSeBA, sodium selenite, or an unsupplemented control on the Se status of in-calf dairy heifers when offered during the latter stages of pregnancy and to determine the Se status of their progeny in the immediate postpartum period.

## MATERIALS AND METHODS

### Animals and Diets

All animals used in this study were maintained at the Centre for Dairy Research, University of Reading (Reading, UK). Animals were kept and experimental procedures undertaken in accordance with the Animals (Scientific Procedures) Act of 1986. A total of 42 in-calf heifers (mean weight: 566.8 ± 4.65 kg; ~170 d of gestation) were recruited for the study. Before study enrollment, animals were managed outside, grazed on grass, and received no supplemental Se. Two weeks before study enrollment, animals were brought into the housing facility for training with Calan gates (individual feeding gates; American Calan Inc., Northwood, NH). Due to the spread in calving dates, enrollment in the study occurred between December 2016 and April 2017. Animals were blocked by weight and calving date and then randomly allocated to 1 of 3 dietary treatments; each treatment comprised 14 animals.

During the course of the study, all animals were fed through individual Calan Broadbent gates (American Calan Inc.) and received a TMR that incorporated a mineral mix (Table 1) that had been manufactured to contain no additional Se; background Se of the basal ration was calculated to be 0.029 mg of Se/kg of DM based on analysis of compositional ingredients for total Se (Table 2). The study comprised a 7-wk wash-out period followed by an 8-wk supplement period. During the wash-out period the TMR was offered to all animals at a rate of 25 g of DM/kg of BW per day in line with standard farm practice to avoid animals becoming overfat during pregnancy, resulting in potential calving difficulties. During the supplementary period animals received access to the same TMR, to which 1 of 3 supplements (Table 3) was top-dressed once daily.

Selenium supplements were mixed and provided by Adisseo France S.A.S (Centre of Excellence and Research in Nutrition, Commentry, France). Treatments used in this study included a negative control (**Con**), HMSeBA, and sodium selenite (**NaSe**). The Con supplement comprised a wheat flour carrier containing no additional Se. The HMSeBA supplement comprised

**Table 1.** Basal TMR ingredients and composition

| Item | Value |
|---|---|
| Ingredient (g/kg of DM) | |
| Maize silage | 143 |
| Grass silage | 540 |
| Straw | 307 |
| Mineral mix | 10 |
| Mineral mix composition (g/kg of product unless noted) | |
| Calcium | 208 |
| Phosphorus | 40 |
| Sodium | 80 |
| Magnesium | 50 |
| Manganese (manganese oxide) (mg/kg) | 5,000 |
| Copper (copper sulfate) (mg/kg) | 2,000 |
| Zinc (zinc oxide) (mg/kg) | 6,000 |
| Cobalt (carbonate) (mg/kg) | 90 |
| Iodine (calcium iodate) (mg/kg) | 400 |
| Selenium | Not included |
| Vitamin A (IU/kg) | 450,000 |
| Vitamin $D_3$ (IU/kg) | 75,000 |
| Vitamin E (IU/kg) | 3,000 |
| Biotin (mg/kg) | 95 |

**Table 2.** Nutritive value of compositional ingredients (mean ± SD)

| Item | Grass silage | Maize silage | Straw |
|---|---|---|---|
| DM (g/kg) | 231 ± 5.6 | 359 ± 34.1 | 899 ± 2.6 |
| ME[1] (MJ/kg of DM) | 10.4 ± 1.45 | 11.7 ± 0.47 | ND[2] |
| CP (g/kg of DM) | 145 ± 13.5 | 73 ± 3.6 | 43 ± 9.2 |
| Ash (g/kg of DM) | 102 ± 1.6 | 33 ± 1.8 | 73 ± 3.6 |
| NDF (g/kg of DM) | 504 ± 65.2 | 350 ± 13.6 | 770 ± 7.2 |
| ADF (g/kg of DM) | 299 ± 44.7 | 184 ± 13.6 | 452 ± 7.2 |
| Se (mg/kg of DM) | 0.049 ± 0.005 | 0.019 ± 0.004 | 0.030[3] |

[1]Predicted ME according to McDonald et al. (2002).
[2]Not determined.
[3]Single determination.

a wheat flour carrier containing HMSeBA (Selisseo, Adisseo France S.A.S.) at 15.9 mg of Se/kg of DM. The NaSe supplement comprised a wheat flour carrier containing sodium selenite at 14.0 mg of Se/kg of DM. The quantity of the HMSeBA and NaSe supplements offered daily was adjusted to equalize dietary Se supply between these 2 Se sources and equated to 2.74 mg of Se/cow per day (Table 3). The average feed intake over the course of the supplementary period of the study was approximately 8.5 kg of DM/cow per day, equating to 0.32 mg of Se/kg of DM feed in Se-supplemented animals.

## Feed Samples

Samples of maize silage, grass silage, and straw were taken for Se analysis (detailed later) before study commencement and at weekly intervals during the study. Samples were stored at −20°C and later pooled by period before analysis (nutritional and Se composition). Samples of mineral mix were taken from each bag when opened, stored at −20°C, and later pooled by period before Se analysis. Samples of the 3 supplements were analyzed for Se concentration before study commencement and again at the end of the study.

Compositional ingredients of the TMR fed during the experimental period were collected weekly, pooled by month, and sent to Scientec Analytical Services (Cawood, North Yorks, UK) for determination of nutritional composition. Briefly, DM, CP, NDF, and ADF were determined using near-infrared spectroscopy using the receiving laboratory's own calibrations. Ash was determined by incinerating the sample at 510°C for 4 h. Metabolizable energy was estimated using the following equation (McDonald et al., 2002):

$$\text{ME (MJ/kg of DM)} = 0.014 \text{ neutral cellulase gammanase digestibility} + 0.025 \text{ oil}.$$

## Animal Performance

Body weight and BCS were recorded at enrollment in the study (start of the wash-out period), at the start of the supplementary period, and again at 2 wk before expected calving date. Feed offered and refused was recorded during the supplementary period until approximately 2 wk before expected calving date. Feed was prepared fresh daily and offered through Calan Broadbent gates (American Calan Inc.). Refusals were measured on Mondays, Wednesdays, and Fridays. Feed consumed was calculated by difference. Dry matter concentration of feed offered and refused was determined weekly by drying samples until a constant weight at 105°C using a force draft oven.

## Blood Sampling and Colostrum

Blood samples were taken weekly from each individual heifer from enrollment until 2 wk before expected

**Table 3.** Treatment designation and proposed level of supplementation required to equalize dietary Se intake between Se-supplemented treatments

| Treatment[1] | Analyzed mean Se concentration (mg/kg of DM) | Supplement supply (g of DM/d) | Supplement Se supply (mg of Se/heifer per day) |
|---|---|---|---|
| Con | 0.020 | 0.172 | 0.004 |
| NaSe | 14.0 | 0.196 | 2.74 |
| HMSeBA | 15.9 | 0.172 | 2.74 |

[1]Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid.

calving date and as soon as possible postcalving. Blood samples were taken from each individual calf as soon as possible after parturition. Blood samples were taken from the tail vein of heifers or the jugular vein of calves by venipuncture into three 10-mL pretreated heparin tubes using the Vacutainer system (BD, Wokingham, Berks, UK). Samples were placed immediately onto ice following collection. One tube was sent, unprocessed, to the Animal and Plant Health Agency (Shrewsbury, Shropshire, UK) for determination of whole-blood glutathione peroxidase (**GSH-Px**) activity using an Olympus AU400 Chemistry Analyzer (Olympus UK, Watford, UK) based on the method of Anderson et al. (1978); packed cell volume was used to convert results to U/mL of red blood cells. The remaining 2 tubes were centrifuged at 1,000 × *g* for 15 min at 4°C in a Heraeus Biofuge Stratos centrifuge (Thermo Fisher Scientific, Basingstoke, UK). The resultant plasma fractions were decanted into labeled tubes, capped, and frozen at −20°C until analysis. Weekly heifer plasma samples were analyzed for total Se. Selenium species (SeMet and SeCys) and malondialdehyde (**MDA**) were determined in heifer plasma samples taken 3 wk before expected calving date. Total Se and MDA concentrations were determined in calf plasma samples.

Colostrum samples were taken from each dam at first milking and frozen at −20°C until analysis. Colostrum samples were analyzed for total Se, Se species (SeMet and SeCys), and IgG concentration. Immunoglobulin G was determined in thawed colostrum samples using a commercially available bovine IgG ELISA kit (ab205078, Abcam, Cambridge, UK).

### Se Analysis

All Se and Se species (SeMet and SeCys) analysis was conducted by UT2A laboratory (Pau, France). Total Se in feeding stuffs and in plasma and colostrum samples was determined according to the method described by Vacchina and Dumont (2018). Briefly, samples were mineralized by a mixture of $HNO_3$ and $H_2O_2$ within a closed-vessel heating block system. The solution was further diluted with water, and Se was subsequently determined using inductively coupled plasma MS (**ICP-MS**; Agilent 7500cx, Agilent Technologies, Tokyo, Japan). The $^{78}Se$ isotope (Analab, Bisheim, France) was used for quantification by the method of standard addition. The collision–reaction cell of the ICP-MS was filled with $H_2$. Samples were determined in duplicate; replicate variation did not exceed 25%.

The selenized AA concentrations of plasma and colostrum were determined by HPLC-ICP-MS using the method described by Vacchina et al. (2018). Briefly, samples were initially incubated with DL-dithiothreitol and iodoacetamide to reduce and alkylate SeCys and subsequently were incubated with a mixture of protease and lipase to digest the proteins. The supernatant was then separated by centrifugation and purified by size exclusion liquid chromatography (cut-off value of the steric exclusion column was 7 kDa). The selenized AA were finally quantified in the purified fraction by reversed-phase HPLC coupled to ICP-MS detection. For this, an Agilent 7500cx ICP-MS (Agilent Technologies) was coupled to a model 1100 HPLC pump (Agilent Technologies, Wilmington, DE) as the delivery system. Quantification was performed using the method of standard addition of SeMet (Sigma-Aldrich, Saint-Quentin Fallavier, France). Samples were determined in duplicate; replicate variation did not exceed 25%.

### MDA Determination

Malondialdehyde concentration in heifer and calf plasma samples was determined by fluorimetry using a thiobarbituric acid reactive substances assay kit (OxiSelect TBARS Assay Kit–MDA Quantitation, Cell Biolabs Inc., San Diego, CA). Briefly, samples or MDA standards were first reacted with thiobarbituric acid at 95°C. After a brief incubation, samples and standards were read fluorometrically at 540 nm excitation and 590 nm emission. The MDA concentration of samples was determined by comparison with a predetermined MDA standard curve with a range of 0 to 125 nmol/mL.

### Statistical Analysis

Data pertaining to heifer performance (BW, BCS, and feed intake), plasma Se, and whole-blood GSH-Px activity were analyzed as repeated measures using the mixed model procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). Factors in the model included animal, treatment, and time point. Animal was used as the repeated subject and time was used as the repeated measure. The covariance structure of the model was compound symmetry. As enrollment in the study was conducted over a period of 4 mo, expected calving date was used as a covariate term. Data pertaining to heifer plasma MDA concentration, the selenized AA concentration of heifer plasma, and colostrum and the total Se and IgG concentration of colostrum, calf plasma total Se, whole-blood GSH-Px activity, and plasma MDA concentrations were analyzed by ANOVA, where treatment was the independent variable in the model. Treatment means were separated using the Tukey simultaneous pairwise comparisons test. Probability values of

**Table 4.** Effect of selenium source on BW, BCS, and feed intake of pregnant heifers

| Item | Treatment[1] | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Con | NaSe | HMSeBA | | |
| BW (kg) | | | | | |
| Enrollment | 560.1 | 565.4 | 574.9 | 8.19 | 0.440 |
| End of wash-out period | 594.9 | 604.7 | 613.7 | 7.67 | 0.234 |
| 2 wk precalving | 629.6 | 640.9 | 641.8 | 7.91 | 0.497 |
| BCS | | | | | |
| Enrollment | 3.1 | 3.2 | 3.3 | 0.07 | 0.524 |
| End of wash-out period | 3.4 | 3.3 | 3.3 | 0.07 | 0.756 |
| 2 wk precalving | 3.4 | 3.4 | 3.4 | 0.07 | 0.885 |
| Feed intake (kg of DM/d) | 8.45 | 8.59 | 8.49 | 0.14 | 0.788 |

[1]Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid.

<0.05 were considered to be statistically significant. Data are presented as least squares means with the standard error of the means.

## RESULTS AND DISCUSSION

There were no effects of treatment on levels of feed intake, BW, or BCS over the course of the study (Table 4). This lack of effect with respect to feed intake, growth performance, and BCS is not surprising as several studies in cattle have reported limited to no response to supplemental Se with respect to intake and growth, especially when the Se status of the animals is not considered to be markedly suboptimal (Gunter et al., 2003; Stockdale et al., 2011; Cortinhas et al., 2012). The Se status of animals from the current study, as indicated by whole-blood GSH-Px activity (Table 5), was above values associated with Se deprivation (Underwood and Suttle, 2010) irrespective of treatment.

Plasma Se declined more rapidly during the wash-out period than whole-blood GSH-Px activity (Table 5, Figure 1). Between enrollment and the end of the wash-out period, irrespective of treatment, plasma Se had declined by approximately 15% of enrollment values, whereas whole-blood GSH-Px activity had declined by approximately 8%. Plasma Se tends to reflect short-term Se status, whereas whole-blood GSH-Px activity provides a better estimation of long-term Se status (Rowntree et al., 2004), with changes mirroring erythrocyte turnover rates (Stowe and Herdt, 1992; Juniper et al., 2008b). This is reflected in changes to plasma Se and whole-blood GSH-Px activities in Con heifers over the course of the current study: plasma Se declined steadily over the course of the entire study

**Table 5.** Effect of selenium (Se) source on whole-blood glutathione peroxidase (GSH-Px) activity, plasma Se and malondialdehyde (MDA) concentrations, and colostrum Se and IgG concentrations of pregnant heifers

| Item | Treatment[1] | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | Con | NaSe | HMSeBA | | |
| Plasma Se (ng/mL) | | | | | |
| Enrollment | 61.6 | 61.2 | 64.3 | 1.78 | 0.576 |
| End of wash-out period | 50.2 | 52.4 | 54.9 | 1.78 | 0.896 |
| 2 wk precalving | 45.3[c] | 61.7[b] | 69.9[a] | 2.23 | <0.001 |
| Calving | 38.9[c] | 58.1[b] | 67.4[a] | 1.78 | <0.001 |
| Whole-blood GSH-Px activity[2] (U/mL of RBC) | | | | | |
| Enrollment | 118.4 | 118.1 | 113.0 | 4.39 | 0.618 |
| End of wash-out period | 109.7 | 107.9 | 103.4 | 2.14 | 0.117 |
| Calving | 93.0[b] | 108.6[a] | 108.4[a] | 2.63 | <0.001 |
| MDA concentration (nmol/mL) | | | | | |
| End of wash-out period | 10.89 | 10.62 | 9.58 | 0.502 | 0.163 |
| 3 wk precalving | 9.24 | 9.02 | 9.25 | 0.572 | 0.950 |
| Calving | 5.70 | 4.86 | 5.71 | 0.382 | 0.211 |
| Colostrum Se (ng/mL) | 53.2[c] | 73.0[b] | 92.4[a] | 4.58 | <0.001 |
| Colostrum IgG (mg/g) | 172.2 | 140.5 | 153.2 | 12.5 | 0.208 |

[a–c]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid.

[2]Unit is defined as the amount of GSH-Px that will cause the oxidation of 1 nmol of NADPH to $NADP^+$ per minute at 25°C. RBC = red blood cells.

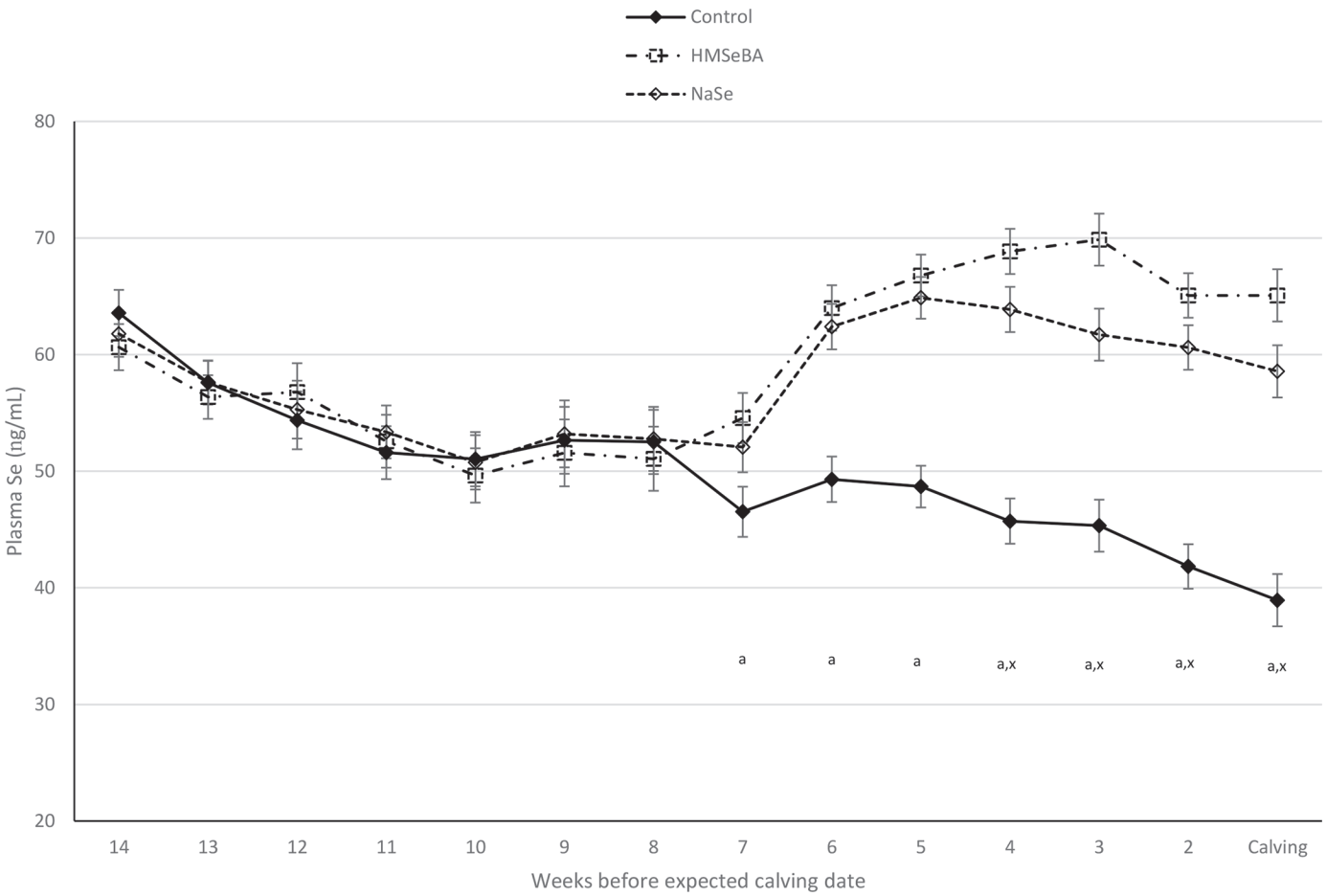


**Figure 1.** Effect of selenium source on the plasma selenium (Se) concentration of growing pregnant heifers (wk 14 to 8 = wash-out period, wk 7 to calving = period of selenium supplementation). NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid. Data are presented as LSM ± SEM. Treatment × time interaction ($P < 0.001$). The letter a denotes time-dependent differences ($P < 0.05$) between supplemented and unsupplemented animals; the letter x denotes time-dependent differences ($P < 0.05$) between Se sources.

(Figure 1), whereas the decline in GSH-Px activity was most notable in Con animals during the last 2 wk of the study (Supplemental Figure S1, https://doi.org/10.3168/jds.2018-16065).

In both HMSeBA and NaSe heifers, plasma Se was seen to increase markedly during the initial period of supplementation. However, 4 wk into the period of supplementation, plasma Se started to decline marginally in NaSe-supplemented heifers; 2 wk later, plasma Se started to decline gradually in HMSeBA-supplemented heifers (Figure 1). This decline in plasma Se may be a consequence of BW-related changes to intake behavior and the fact that both Se supplements were fed at a fixed level throughout the period of supplementation (2.74 mg of Se/heifer per day). Although animals had a mean feed intake of 8.5 kg of DM/d during the supplementary period, absolute intakes at the start of the supplementary period were lower than those recorded 2 wk before calving and increased in line with changes to BW as the study progressed. Irrespective of treatment and throughout the supplementary period, intakes expressed as a function of BW were 14 g of DM/kg of BW. Given the fixed level of Se supply during the period of supplementation, Se intakes, if expressed as a function of BW, effectively declined, and the reductions seen in plasma Se at calving are proportional to this effective decline in Se supply.

Despite this, at 2 wk precalving and at calving, plasma Se concentrations were greater in Se-supplemented heifers compared with Con heifers, with concentrations being higher in HMSeBA heifers compared with NaSe heifers ($P < 0.05$). Conversely, changes to whole-blood GSH-Px activity in Se-supplemented heifers during the period of supplementation were minimal, and as such there were no differences in GSH-Px activity between the 2 Se supplements at calving. However, GSH-Px activity of Con heifers was seen to decline appreciably around the time of calving and was markedly lower

than that seen in the 2 Se-supplemented groups ($P < 0.05$). Reduced GSH-Px activity in the peripartum period has been reported by Colakoglu et al. (2017); however, these authors did not use any Se intervention, nor did they report on the Se status of the animals in their study. These authors also reported increased plasma MDA concentrations during the peripartum period, whereas MDA concentrations in the current study were seen to decline. Increased GSH-Px mRNA expression has been demonstrated in situations where oxidative stress has been simulated (Sneddon et al., 2003; Touat-Hamici et al., 2014), and this upregulation is independent of Se status (Sunde and Raines, 2011). However, GSH-Px activity is dependent on Se status. The reductions seen in GSH-Px activity in the control group of the current study and that reported by Colakoglu et al. (2017) may reflect suboptimal Se supply, thus emphasizing the importance of an adequate Se supply during the peripartum period. Although the current study did not establish any differences between sources with respect to GSH-Px activity in heifers, the study of Sun et al. (2019) in which mid-lactation dairy cows were subjected to heat stress reported that during periods of induced heat stress animals that had received HMSeBA rather than selenite had reduced indicators of oxidative stress and elevated GSH-Px activity. This would suggest that GSH-Px activity can be upregulated during times of oxidative stress and that organic Se forms help alleviate the adverse effects associated with this stress.

The determination of selenized AA in plasma taken 3 wk precalving (Figure 2) intimates an explanation for the different responses seen between treatments with regard to plasma Se and whole-blood GSH-Px activity. The SeMet concentration of plasma was similar between NaSe and Con heifers but greater in the plasma of HMSeBA heifers ($P < 0.05$), whereas the SeCys concentration of plasma was similar between NaSe and HMSeBA heifers but lower in Con heifers ($P < 0.05$). This suggests that the higher plasma Se in HMSeBa heifers compared with NaSe heifers was a consequence of SeMet incorporation and that the difference between NaSe heifers and Con heifers was attributable to SeCys. Furthermore, the similarity between the sum of SeMet and SeCys in plasma and total Se in plasma suggests a complete conversion of HMSeBA to SeMet in plasma.

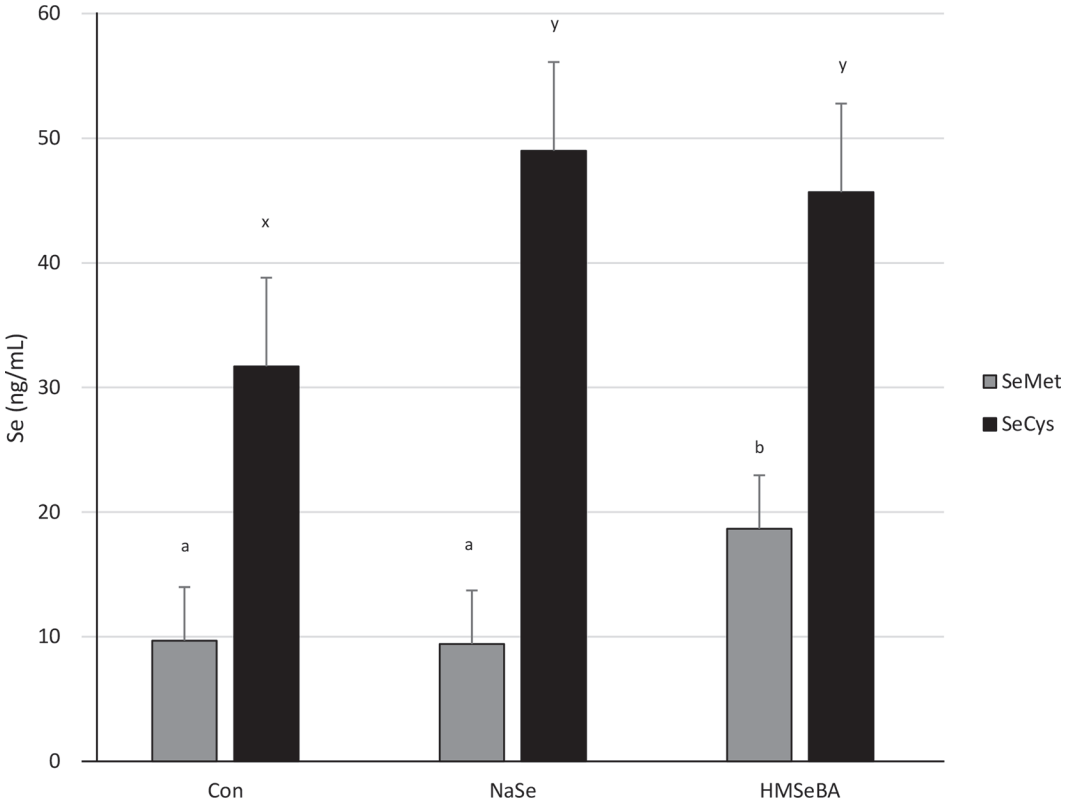


**Figure 2.** The effect of selenium (Se) source on the concentration of selenomethionine (SeMet) and selenocysteine (SeCys) in the plasma of pregnant heifers 3 wk before calving. Data are presented as LSM ± SEM. Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid. Different letters (a, b) denote differences ($P < 0.05$) between treatments in SeMet; different letters (x, y) denote differences ($P < 0.05$) between treatments in SeCys.

**Table 6.** Effect of dam selenium (Se) source on calf whole-blood glutathione peroxidase (GSH-Px) activity and plasma Se and malondialdehyde (MDA) concentrations

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | Con | NaSe | HMSeBA | SEM | *P*-value |
| Plasma Se (ng/mL) | 31.2[c] | 39.2[b] | 44.8[a] | 1.45 | <0.001 |
| Whole-blood GSH-Px[2] (U/mL of RBC) | 79.3[b] | 94.4[a] | 98.5[a] | 2.81 | <0.001 |
| Plasma MDA (nmol/mL) | 4.99 | 6.03 | 5.23 | 0.601 | 0.460 |

[a–c]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid.

[2]Unit is defined as the amount of GSH-Px that will cause the oxidation of 1 nmol of NADPH to $NADP^+$ per minute at 25°C. RBC = red blood cells.

This accumulation of SeMet in the plasma and blood of cattle receiving organic Se sources has been reported previously (Juniper et al., 2006, 2008b; Phipps et al., 2008) and is attributable to the better bioavailability and nonspecific incorporation of dietary SeMet into the protein pool. The lower SeCys concentration of Con heifers ($P < 0.05$) and the lack of difference between NaSe and HMSeBA heifers tends to reflect treatment GSH-Px activities at calving. The synthesis of selenoproteins is hierarchal, and as such the expression of some selenoproteins is very much dependent on Se supply (Howard et al., 2013; Seyedali and Berry, 2014). However, previous work has shown that the SeCys concentration of blood and plasma is generally unaffected when supranutritional levels of dietary Se (5.6 mg of Se/kg of DM) are fed that are well above the animal's Se requirement (Juniper et al., 2008a). This reflects the highly regulated production of selenoproteins because there is no free pool of SeCys in mammals and only newly synthesized SeCys is incorporated into selenoproteins (Surai, 2006).

Plasma Se concentration of calves born to Con heifers was lower than that of calves born to heifers that had received Se supplements ($P = 0.001$) and was greater in calves from HMSeBA-supplemented heifers than in calves from NaSe-supplemented heifers (Table 6; $P < 0.05$). This enhanced Se status of calves born to dams that received organic Se supplements during the latter stages of pregnancy has been reported previously (Pehrson et al., 1999; Gunter et al., 2003; Guyot et al., 2007) and is most likely attributable to SeMet transfer from the dam to the calf. Selenoprotein-based transfer mechanisms have been identified in the mouse placenta; these ensure an adequate Se supply to the developing fetus, especially when maternal Se supply is poor (Burk et al., 2013). However, the transport of SeMet across the placenta is unregulated and is likely to be dependent on maternal SeMet supply (Burk et al., 2013). Under conditions of limited dietary Se supply, SeMet transfer may be limited, and as such Se is likely to be supplied to the fetus through previously mentioned selenoprotein transfer mechanisms (Burk et al., 2013). Although the selenized AA of calf plasma immediately postpartum was not determined in the current study, and assuming that the bovine placenta functions in a similar manner as the murine placenta with regard to selenoprotein and SeMet transfer, it is probable that the SeMet concentration of plasma from calves born to HMSeBA heifers was greater than that of calves born to Con and NaSe heifers.

Calf whole-blood GSH-Px activity was lower in calves born to Con heifers than in calves born to those that received Se supplements ($P = 0.001$), although there were no differences between calves born to HMSeBA and NaSe heifers despite the higher plasma Se of calves born to HMSeBA-supplemented dams. However, calf GSH-Px activities, irrespective of treatment, were greater than activities that are associated with Se deprivation (Underwood and Suttle, 2010). Furthermore, the lack of any difference in calf plasma MDA concentrations with respect to their dam treatments suggests that differences seen in calf GSH-Px activities between those born to dams supplemented with Se and those born to those that were not supplemented had a minimal effect on levels of lipid peroxidation.

The Se concentration of colostrum was predictably greater in heifers that had received Se supplements (Table 5; $P < 0.01$) and greatest in those that had been supplemented with HMSeBA ($P < 0.05$). This difference in response between comparable doses of HMSeBA and NaSe has been reported previously in the milk of mid-lactation dairy cows (Sun et al., 2017). Although Se speciation was not conducted on milk produced in the study of Sun et al. (2017), several studies have shown that differences in milk Se responses between comparable doses of organic and inorganic Se supplements are usually a consequence of SeMet incorporation (Phipps et al., 2008; Calamari et al., 2010), and studies in monogastric species have shown that increases in tissue Se as a response to HMSeBA supplementation are primarily

a consequence of the incorporation of SeMet (Briens et al., 2014; Jlali et al., 2014). However, Se speciation analysis of colostrum from the current study (Figure 3) showed that the marked difference in the total Se concentration in colostrum from HMSeBA-supplemented animals was attributable to SeCys rather than SeMet. This finding was unexpected because it was anticipated that SeMet would have been the predominant selenized AA given the findings of earlier studies feeding organic forms of Se to dairy cattle (Phipps et al., 2008; Calamari et al., 2010), where SeMet was the predominant selenized AA. The Se concentration of colostrum has been reported to be markedly greater than that of following milk in several species (Slavik et al., 2008; Peters et al., 2010; Meyer et al., 2011; Salman et al., 2013), although very few if any studies have reported on the selenized AA concentration of colostrum. The reason for the difference between colostrum and milk total Se concentration remains unclear and may simply reflect differences in milk secretory processes pre- and postpartum. However, the higher Se concentration of colostrum may confer benefits to the newborn in terms of the conferment of passive immunity; Apperson et al. (2018) reported that passive intestinal transfer of ovalbumin was improved in calves born to Se-supplemented dams.

As previously mentioned, SeCys concentrations in plasma tend to be correlated with selenoprotein expression and activity in blood, and it is likely that elevated concentrations of SeCys in milk are indicative of the selenoprotein concentration of colostrum. Hill et al. (2014) reported that selenoprotein P was the major Se transport protein in mouse milk and postulated that this may be an important mechanism in transferring maternal Se to the nursing neonate. If the elevated SeCys concentration of colostrum reported in the current study was indicative of selenoprotein P concentration, then it could be hypothesized that the mechanisms that are responsible for IgG transfer in the newborn calf might also be involved in the uptake and transfer of Se as selenoprotein P in the immediate neonatal period. The advantages of this mechanism are that selenoprotein P could be transferred to the systemic circulation in very early life, thus conferring benefits to the newborn with respect to Se status. However, neither the selenoprotein P concentration of milk nor calf serum

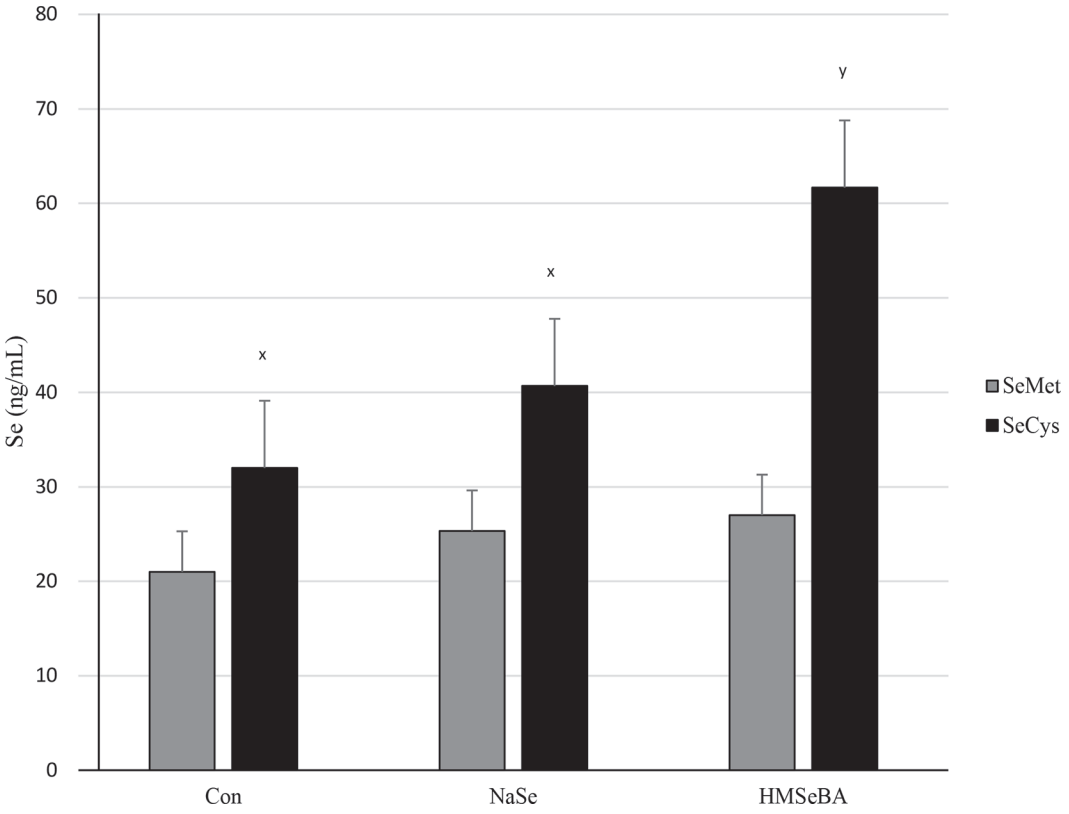


**Figure 3.** The effect of selenium (Se) source on the concentration of selenomethionine (SeMet) and selenocysteine (SeCys) in the colostrum of recently calved heifers. Data are presented as LSM ± SEM. Con = control; NaSe = sodium selenite; HMSeBA = 2-hydroxy-4-methylselenobutanoic acid. Different letters (x, y) denote differences ($P < 0.05$) between treatments in SeCys.

before or after suckling were determined in this study, and as such it is not possible to speculate further on this hypothesis.

Dam Se supply had no effect on the IgG concentration of colostrum (Table 5). Previous studies have demonstrated that milk IgG concentration can be lower in cattle that have marginal Se deficiency (Swecker et al., 1995). However, as previously mentioned, the Se status of the animals in the current study would not be considered deficient and as such responses in milk IgG concentration would not be anticipated.

## CONCLUSIONS

The results of this study demonstrate the benefits of Se supplementation with regard to the Se status of the dam and her calf, to the administration of Se supplements in the latter stages of pregnancy, and to the form of Se supplement. Selenium status of both the dam and calf was further improved by the administration of the SeMet precursor HMSeBA compared with the mineral form NaSe. This improvement in Se status is likely a consequence of SeMet supply, uptake, and incorporation, as SeMet was seen to be greater in the plasma of HMSeBA-supplemented animals. However, increases in colostrum Se of HMSeBA-supplemented animals appear to be more a consequence of elevated SeCys than SeMet. This might be suggestive of a mechanism that improves Se supply, uptake, and assimilation in the neonate during the immediate postpartum period.

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